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Dear Dr. Shelby:

The following comments are submitted on behalf of the 800,000 members and supporters of People for the Ethical Treatment of Animals (PETA), in response to the draft National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) Expert Panel's report on the reproductive and developmental toxicity of acrylamide, which was prepared on March 15, 2004. PETA is the world's largest animal rights organization and is committed to using the best available science to protect animals from suffering and to promoting the acceptance of alternatives to animal testing.

Our general comments concern the NTP's system for soliciting public comment on proposed toxicology studies. In the NTP-CERHR program, the Expert Panel prepares a draft report which does not state the toxicology studies that are to be proposed. This draft report is available for public comment, after which an expert meeting is held, and the final report is prepared. This is an unreasonable approach since it means that interested individuals and organizations, such as PETA, are forced to guess from the draft report what studies are likely to be required. In the EPA's HPV program, in contrast, corporations propose test plans which are posted online in order to allow for comments to be made by the EPA and the interested public, after which the corporations prepare final test plans. The NTP's current approach appears to disregard public input while requesting it, and we therefore urge the NTP to consider a different and better approach.

We must also point out that toxicology studies on acrylamide were recommended by the NTP as recently as July 2003. At that time, the NTP recommended studies on the toxicity characterization, toxicity mechanism, toxicokinetics, carcinogenicity, and bioavailability (Dept. of Health and Human Services 2003), and we submitted detailed comments on these recommendations in September 2003 (Seidle 2003). We cannot understand why the NTP-CERHR has decided to assess the need for additional toxicology studies so soon after that original recommendation.

Our specific comments on the acrylamide draft report are as follows:

The Expert Panel is to be commended for having surveyed a large number of previous studies. At least 18 developmental toxicity studies and 17 reproductive toxicity studies have been carried

out in animals (draft report, pp. 91-148) and the results of these studies, together with assessments of their relevance and adequacy, are presented in the draft report. At the same time, it appears that the Expert Panel is unjustifiably criticizing previous studies for not providing sufficient information on the methods used, statistical analysis, etc. (see "strengths/weaknesses" sections of the report). For example, the draft report states: "Methods for summarizing fetal findings are poorly described" (p. 97); "This paper is of limited utility due to the … lack of detail" (p. 108); "a real paucity of specifics about study conduct and findings significantly limits the ability of the Panel to use this study" (p. 116); "The lack of specifics and details moderate our certainty that acrylamide produces no effect on female reproductive function" (p. 117); "a lack of detail of the methods used (it would be impossible to reproduce the studies here based on the methods given in this paper)" (p. 120). Yet most of this information is probably available, and was simply not published because it was not relevant to the specific research. With some minimum effort this information could probably have been obtained by contacting the researchers responsible for the publications.

It is not clear from the draft report's conclusions (pp. 114, 147) whether the CERHR is likely to judge that further animal experiments are required. However, the central conclusions are that acrylamide has the following effects: (i) developmental toxicity in rats with maternal oral doses of 4-5 mg/kg/day; (ii) developmental toxicity in mice with paternal intraperitoneal doses of 10-25 mg/kg/day; and (iii) male reproductive toxicity in mice with oral doses of 13 mg/kg/day. As these conclusions are based on data from a large number of studies, and many of these have been judged by the CERHR to meet its requirements for specific areas (pp. 93, 104, 108, 120, 121, 123, 125, 128, 130, 132, 139), it is reasonable to conclude that rodents show developmental and reproductive toxicity with acrylamide doses of 5 mg/kg/day or higher. To the extent that animal data are of any value for predicting human toxicity, we therefore consider the research to have been effectively completed. Further animal studies are only likely to provide data of academic interest, in areas such as the mechanism of acrylamide toxicity in rodents. If such studies are requested and carried out, they should be recognized as being pure research, and should not be approved under the guise of being relevant to the prevention of human toxicity.

In addition, acrylamide toxicity shows marked interspecies variation. For example, the Expert Panel has noted that approximately ten times the maternal gavage dose is needed to give rise to developmental toxicity in rats than is needed for similar effects in mice (p. 114). This finding is consistent with the fact that mice are approximately ten times more sensitive than rats to the carcinogenic effects of acrylamide (Paulsson 2001). Since rats and mice are much more closely related than either is to humans, the rodent data have little or no value for estimating the level of exposure needed to give rise to reproductive and/or developmental toxicity in humans. There are also two other factors suggesting that there are both qualitative and major quantitative interspecies differences in toxicity. First, some of the mechanisms that have been suggested for acrylamide developmental toxicity are particularly likely to vary between species:

(a) The authors of one report have suggested that the increases in the numbers of rats and mice born with extra ribs as a result of maternal acrylamide administration may have been due to "the stress of treatment rather than chemical-specific fetotoxicity" (p. 98). Clearly, such stress, with major psycho-social components, is liable to differ markedly and unpredictably between species.

(b) Much of the developmental toxicity that has been studied has been in the nervous system (pp. 102-106) and neurotoxicity is especially prone to differ among species (US Congress 1990). Furthermore, with respect to neurotoxicity, humans show more heterogeneity than the species used in laboratories (WHO 1978). For example, several studies of acrylamide non-developmental neurotoxicity suggest that susceptibility changes with age (pp. 81-83).

Second, to the limited extent that acrylamide metabolism is understood, it shows major interspecies variability, as detailed below. This variability is highly important because at least some of the toxicity of acrylamide is due to its metabolites (Calleman 1996); for example, glycidamide is probably responsible for acrylamide genotoxicity (Paulsson 2001). However, the proportion of acrylamide toxicity due to its metabolites is unknown, so the effects of the following interspecies differences cannot be predicted:

(a) Two groups of pathways are thought to be involved in acrylamide metabolism: via glutathione conjugation, which is catalyzed by glutathione-S-transferase; and via glycidamide, by reaction with cytochrome P450 (Calleman 1996, Friedman 2003). However, there are interspecies differences in the proportions of acrylamide metabolized by the two pathways. For example, mice have been shown to metabolize a far larger proportion of acrylamide via the glycidamide pathway than do rats (Sumner 1992, 2003), and the proportions of acrylamide metabolized by these pathways in humans differ from those in both rats and mice (Bergmark 1993). In addition, in rats the proportions of these metabolites are independent of the route of acrylamide administration, whereas in mice the proportion of metabolism via the glutathione conjugation is considerably higher when administration is by gavage than when it is by inhalation (Sumner 2003), suggesting the probability of additional unpredictable interspecies differences.

(b) Glutathione conjugation, in the first pathway, is catalyzed by glutathione-S-transferase (p. 23). However, glutathione-S-transferase is highly polymorphic, and the different forms probably differ greatly between mammalian species (pp. 83-84). These forms differ in the efficiency of conjugation, and may also differ with respect to the precise pathways catalyzed (p. 23).

(c) The reaction with cytochrome P450, in the second pathway, is catalyzed in mice by the enzyme cytochrome P450 2E1 (CYP2E1). However, the CERHR merely states that it is "reasonable to assume that CYP2E1 is the isoform that biotransforms acrylamide in humans" (p. 90), providing no evidence for this supposition. In addition, cytochrome P450 levels vary widely between individual humans, as a result of polymorphism, and wide variations can therefore also be expected between species (p. 90). Furthermore, the post-glycidamide section of this pathway is known to follow at least three different branches (p. 22), and there are no grounds for assuming these to be of equal importance in different species.

For the above reasons, the Expert Panel appropriately concludes that "the current information is insufficient for predicting the effects of variable metabolic enzyme activity on acrylamide-induced toxicity" (p. 90).

In addition to interspecies variability in metabolism, there is known to be marked interspecies variability in bioavailability. For example, serum levels of glycidamide-hemoglobin adduct per unit dose of acrylamide have been shown to be 3-10 times higher in mice than in rats (Paulsson 2001), and when radioactive acrylamide was administered, the total recovered radioactivity in mice was 2.8 times that in rats (Sumner 2003). There are also marked interspecies differences in acrylamide elimination rates, with the rate in humans probably being at least five times that in rats (Calleman 1996).

Therefore, to conclude with respect to interspecies variability, there are no grounds for supposing that data on the developmental or reproductive toxicity of acrylamide in one species are relevant for other species. The Expert Panel tacitly acknowledges this, and repeatedly states that rodent data are merely "assumed" to be relevant to humans (pp. 114, 147).

We appreciate the fact that acrylamide toxicity has the potential to give rise to major human health problems. Since further animal studies are unlikely to provide useful data, we would like to suggest alternative approaches. Fortunately, an expert committee organized by the WHO and the United Nations' Food and Agriculture Organization (FAO) has outlined the three most important areas for future research ("Acrylamide in food" 2002). These areas, none of which are likely to involve animal experiments, are as follows:

1. Epidemiology

With respect to acrylamide, the WHO/FAO expert committee states that there is a need for "epidemiological studies of relevant cancers in humans" ("Acrylamide in food" 2002). There is an even greater need for epidemiology studies of developmental and reproductive toxicity, because no epidemiology studies whatsoever covering these types of toxicity have been carried out. The only type of occupational acrylamide toxicity to have been epidemiologically verified is neurotoxicity, which has been clearly demonstrated by epidemiology studies in Chinese factory workers subjected to very high exposure (He 1989, Bergmark 1993, Calleman 1994). In addition to this series of studies, there have been three occupational epidemiology studies on the carcinogenicity of acrylamide in factory workers (Sobel 1986, Collins 1989, Marsh 1999), and also two small questionnaire-based dietary epidemiology studies on the carcinogenicity of fried potatoes (Mucci 2003, Pelucchi 2003). These cancer epidemiology studies all yielded essentially negative results, but the conclusion that neither occupational nor dietary acrylamide exposure increases the cancer rate is open to criticism because of the difficulties associated with all these studies (Granath 2001, 2003, Marsh 2001, Schulz 2001, Reynolds 2002). Therefore, as stated by the WHO/FAO expert committee, there is a pressing need for full-scale exposure and epidemiology studies, even with respect to cancer, and far more so with respect to reproductive and developmental toxicity. In particular, because the main mode of exposure to the general population is probably in fried foods (pp. 4-6), a large-scale, international dietary epidemiology study is clearly needed. The dietary acrylamide content must be actually measured, in contrast to the estimates used in the studies by Mucci and Pelucchi. Reynolds, for example, has recommended that retrospective studies be conducted, comparing the changes that occurred when boiled potatoes were replaced as the staple diet

by fried potatoes across much of northern Europe and, secondly, geographic studies should be conducted which compare populations in which the consumption of fried food differs (2002). We must emphasize that we consider human exposure and epidemiology to be the central requirements for research on acrylamide toxicity, and we are disappointed at how little attention the Expert Panel has paid to this area (pp. 6-11).

2. Investigation of acrylamide formation during cooking

The WHO/FAO expert committee emphasizes the need to determine "how acrylamide is formed during the cooking process" ("Acrylamide in food" 2002). Surprisingly, the fundamental chemistry of acrylamide formation seems scarcely to have been investigated, and there is only one paragraph on this issue in the draft report (p. 4). The acrylamide formation mechanism has important implications for health policy. For example; if more acrylamide is formed by frying potatoes and other foods in animal fats more than in vegetable oils, this will provide a reason (in addition to the various other health reasons) for encouraging individuals, food companies, and restaurants to cook using vegetable oils instead of animal fats. The acrylamide content of fried potatoes has been found to vary very widely, from 170 to 12,000 _g/kg (Friedman 2003), and research to elucidate the cause of this variation is therefore essential.

3. Cross-cultural research

The WHO/FAO expert committee states that there is a need for "studies of acrylamide in other foods, including those present in non-European and North American diets" ("Acrylamide in food" 2002). Some non-Western traditional diets, especially those that include fewer animal foods, are widely considered to be healthier than the modern European and North American diet, and we strongly support research into whether this general higher level of healthiness extends to a lower acrylamide content.

In addition to the areas suggested by the WHO/FAO expert committee, more attention should be given to policy issues. There does not appear to be any point in obtaining data about exposure, epidemiology or toxicology, unless there have been prior decisions about the legal, technical and administrative responses that would be necessitated by various possible sets of results. The current concern about acrylamide dates from 1997, when 1,400 tons of an acrylamide-containing sealant were injected into cracks in the rock during the construction of a new railway tunnel in Sweden, causing severe neurotoxicity among the workers as well as massive contamination of the local groundwater. This resulted in cattle being paralyzed and dying, and fish dying in ponds, and this lead to the destruction of all meat, vegetables, fish and dairy products produced in the area as unfit for consumption (Reynolds 2002). Acrylamide has long been known to be toxic, therefore this incident, which sparked outrage among labor and environmental groups, was due to either stupidity or negligence on the part of the construction company. Prevention of such incidents is clearly the responsibility of politicians, lobbyists, lawyers and organized labor, rather than scientists.

Even in the case of routine occupational exposure, at much lower levels than were reached in the tunnel incident, it is questionable whether clear demonstration of human developmental and

reproductive toxicity would in fact lead to increased protection for workers. We suspect that, in many cases, the demand for more data is little more than a smokescreen. Our reason for this claim is that it has long been known that grouters are at risk of neurotoxicity and other toxicity due to using acrylamide-containing grouts, even if they also use personal protective equipment. However, despite repeatedly trying to ban these grouts for 11 years starting in 1991, the EPA finally gave up in 2002 and claimed that affordable protective equipment that sufficiently limits exposure was available (although without any information as to whether this equipment would be likely to be used). The EPA's decision was taken even though acrylamide-containing grouts are manufactured in the USA by only one company (Avanti), and the fact that non-acrylamide-containing substitutes are available ("EPA calls for a ban …" 1998, EPA 2002). A certain degree of skepticism is therefore warranted as to the likely benefits for workers' health of more toxicology data on acrylamide.

With respect to acrylamide in food, it would probably be almost impossible to reduce the acrylamide content without major changes in diet, such as a marked reduction in the consumption of fried food. In this context, McCaffree has made the following comment: "even if acrylamide definitely was proven to cause cancer in humans, how can we get it out of food?" (2003). However, as convenience foods, fried foods, and foods containing animal fats are widely accepted as having a range of serious negative effects on health, attempts should be made to reduce their consumption, quite apart from the effects of acrylamide, and acrylamide data may therefore be superfluous. In this context, Granath has made the following point: "the high consumption of foods such as potato chips or french fries should be avoided for other and more prominent health-related reasons, such as cardiovascular disease" (2003).

Finally, if the CERHR judges that experimental studies are required, the potential for interspecies variability in the mechanism of toxicity means that the best approach to obtaining data about human toxicity will be the use and/or development of *in vitro* test methods. In the case of embryotoxicity, one *in vitro* method that is already available is the rodent embryonic stem cell test, which has been validated by the European Centre for the Validation of Alternative Methods (Genschow 2002).

Please feel free to contact me at 757-622-7382, ext. 8001, or via e-mail at JessicaS@peta.org if you have any questions.

Sincerely,

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